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Transcriptome analysis of flax seeds and capsules

Povkhova L.V.[1, 2]*, Novakovskiy R.O.[1], Rozhmina T.A.[1, 3], Dvorianinova E.M.[1, 2], Frykin R.I.[1, 4], Pushkova E.N.[1], Dmitriev A.A.[1], Melnikova N.V.[1]

[1] *Engelhardt Institute of Molecular Biology, RAS, Moscow, Russia*

[2] *Moscow Institute of Physics and Technology, Moscow, Russia*

[3] *Federal Research Center for Bast Fiber Crops, Torzhok, Russia*

[4] *Lomonosov Moscow State University, Moscow, Russia*

* *povhova.lv@phystech.edu*

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Motivation and Aim: Seeds of flax (*Linum usitatissimum* L.) are a rich source of unsaturated fatty acids (linolenic, linoleic, and oleic acids) and lignans. Linseed varieties differ significantly in fatty acid and lignan content. The pathways of lignan and fatty acid biosynthesis are known to include several key genes, however, molecular mechanisms of their regulation have yet to be understand. This work aims to identify the diversity of molecular mechanisms that determine the fatty acid composition of linseed oil and lignan content in flax seeds. An effective approach to resolve multiple issues of fatty acid and lignan synthesis is transcriptomic studies of different flax varieties with contrasting fatty acid and lignan content. Here, we perform such an analysis for several representative flax varieties at different stages of seed development.

Material and Methods: Cultivars and lines for the research were obtained from the collection of the Institute for Flax (Torzhok, Russia). RNA was extracted independently from seeds and capsules using the Quick-RNA Miniprep Kit (Zymo Research, USA). The concentration and quality of the isolated RNA were assessed using a Qubit 4 fluorometer (Thermo Fisher Scientific, USA) and 2100 Bioanalyzer (Agilent Technologies, USA). cDNA libraries for transcriptome sequencing were prepared using the QIAseq Stranded mRNA Select Kit (Qiagen, USA). The quality of the cDNA libraries was assessed with 2100 Bioanalyzer, and high-quality libraries were sequenced on the Illumina platform.

Results: We studied a representative set of flax varieties, covering the diversity of this agriculture in terms of the fatty acid composition of oil and the content of lignans in seeds. Nine varieties of flax were grown in climatic chambers under three different temperature and watering conditions: control ones; low temperatures and abundant watering; high temperatures and poor watering. For the analysis, we used only typical plants (at least 10 for each cultivar/line) selected using the data on key polymorphisms obtained by targeted sequencing or Cleaved Amplified Polymorphic Sequence (CAPS) analysis. This step helped to avoid erroneous results due to varietal heterogeneity, which is extremely important for an experiment to be reliable. The capsules and seeds were gathered on the 3rd, 7th, 14th, 21st, and 28th day after flowering. We performed a transcriptome sequencing of 270 cDNA libraries: 9 cultivars/lines, 3 temperature/watering modes, 2 types of material (capsules and seeds), 5 collection periods). We analyzed the changes in gene expression profiles during the development of flax seeds and revealed genes with differential expression between varieties, development stages, and growth conditions.

Conclusion: The obtained results will make it possible to identify the diversity of mechanisms determining the fatty acid composition of linseed oil and lignan content in flax seeds, suggest molecular markers associated with the seed characteristics, and develop recommendations for their use in marker-assisted flax selection for the use in food, pharmaceutical, or other industries.

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